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Pharmacokinetic/pharmacodynamic evaluation of marbofloxacin in the treatment of *Haemophilus parasuis* and *Actinobacillus pleuropneumoniae* infections in nursery and fattener pigs using Monte Carlo simulations

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This study evaluated the theoretical clinical outcome of three marbofloxacin posology regimens in two groups of pigs (weaners and fatteners) for the treatment of *Actinobacillus pleuropneumoniae* (App) and *Haemophilus parasuis* (Hp) infection and the appearance of resistant bacteria due to the antibiotic treatment. The probability of target attainment (PTA) for pharmacokinetic/pharmacodynamics (PK/PD) ratios associated with clinical efficacy and with the appearance of antimicrobial resistance for fluoroquinolones at each minimum inhibitory concentration (MIC) or mutant prevention concentration (MPC) were calculated, respectively. The cumulative fraction of response (CFR) was calculated for the three posology regimens against App and they ranged from 91.12% to 96.37% in weaners and from 93% to 97.43% in fatteners, respectively. In the case of Hp, they ranged from 80.52% to 85.14% in weaners and from 82.01% to 88.49% in fatteners, respectively. Regarding the PTA of the PK/PD threshold associated with the appearance of antimicrobial resistance, results showed that marbofloxacin would prevent resistances in most of the animals up to the MPC value of 1 μg/mL.

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INTRODUCTION

Marbofloxacin is a third generation fluoroquinolone widely used in veterinary medicine. Its properties of rapid absorption, good distribution and broad spectrum against most of the swine respiratory pathogens, such *as Haemophilus parasuis* (Hp) and *Actinobacillus pleuropneumoniae* (App), make it a good candidate to deal with a respiratory outbreak due to any of these pathogens.

The major issues of practitioners when treating a large population of animals are to maximize the likelihood of a favourable clinical outcome at population level and to minimize the appearance and development of antimicrobial resistance that could affect future treatments. Pharmacokinetic (PK) and pharmacodynamic (PD) models are a useful tool to foresee clinical efficacy and it could be also used to design and choose the right antimicrobial therapy (Mckellar *et al.*, 2004).

Currently, a great amount of information is available on the pharmacokinetics and pharmacodynamics of fluoroquinolones and the relationship between PK and PD parameters that could be associated with the clinical outcome. The ratios between the area under the curve during the first 24 h and the minimum inhibitory concentration ($AUC_{0\text{-}24}$/MIC) and between the maximum concentration and MIC (C_{max}/MIC) correlate well with successful therapeutic resolution when fluoroquinolones are used to cope with an infection. Thus, a threshold of $AUC_{0\text{-}24}$/MIC of >125 h and C_{max}/MIC of >10 would correlate with successful therapeutic outcome according to literature (Toutain *et al.*, 2002; Mckellar *et al.*, 2004) for fluoroquinolones. Nevertheless, these ratios would not be the most appropriate for the prediction of bacterial resistance. In this case, a better marker to describe the likelihood of appearance of antimicrobial resistance is the ratio between the $AUC_{0\text{-}24}$ and the mutant prevention concentration (MPC) (Zhao & Drlica, 2008). Regarding $AUC_{0\text{-}24}$/MPC, the study of Cui *et al.* (2006) established that a value of $AUC_{0\text{-}24}$/MPC above 25 h restricts the acquisition of resistances in a *Staphylococcus aureus* infection (a gram-positive bacterium). Similar values were found by Olofsson *et al.* (2006), in an *in vitro* study, and Ni *et al.* (2013), in an *in vivo* study (rabbit model), where a ratio $AUC_{0\text{-}24}$/MPC>22 h and >20 h were

established to prevent resistance appearance in the case of a *Escherichia coli* infection (a gram-negative bacteria), respectively.

The use of Monte Carlo simulation (MCS) takes into account the variability of the drug PK and the probability distribution of the bacterial MIC to make predictions of the likely result of different therapeutic approaches, using different antimicrobial dosage regimens. To achieve this goal, it is taken into account the threshold values for PK/PD parameters that correlate with clinical efficacy (Roberts *et al.*, 2011). Thus, MCS could be a useful tool to assess and foresee the probability of a favourable outcome of an antibiotic treatment in a large population of animals. This same approach could be used to foresee the appearance of the antimicrobial resistance taking into account the threshold values for PK/PD parameters associated with this event.

The main objective of this work was to evaluate the usefulness of three marbofloxacin posology regimens against Hp and App taking into account their PK and PD variability. Thus, it was assessed the probability of achieving the threshold PK/PD parameters associated with clinical efficacy and with the appearance of antibiotic resistance for marbofloxacin in two pig groups (weaners and fatteners) usually treated with this antibiotic.

MATERIALS AND METHODS

Marbofloxacin PK data and dose selection

Marbofloxacin PK data for weaner and fattener pigs have been recently published by Schneider *et al.* (2014) and this information have been used with permission of the authors for this research work. Briefly, marbofloxacin pharmacokinetic parameters were determined using compartmental analysis with the WinNonlin software version 5.0.1 (Pharsight Corporation, St Louis, MO. USA) in 10 weaners and seven fattener pigs. Pharmacokinetic profile fitted better to a bicompartmental and monocompartmental model for weaners and fatteners, respectively. One animal was excluded from the weaners due to abnormal values for the simulations.

To carry out the pharmacokinetic and pharmacodynamic evaluation, the doses of 2, 4 and 8 mg/kg for marbofloxacin were selected according to the most frequent posology regimens in use under field conditions. As previously commented, the pharmacokinetic data came from a previous study with a dose of 8 mg/kg bw and the data for the 2 and 4 mg/kg bw doses were inferred taking into account the marbofloxacin dose proportionality as described by Schneider *et al.* (2014). It must be highlighted that these doses are usually applied in different posology regimens in daily practice (2 mg/kg bw three times each 24 h, 4 mg/kg bw twice each 48 h and 8 mg/kg bw in one single shot).

Microbiological data

MIC distribution. MIC distribution was extracted from a poster communication presented at the 4th European Symposium of Porcine Health Management (ESPHM) in 2012 (Giboin *et al.*, 2012). MIC was determined as explained elsewhere (Meunier *et al.*, 2004; Kroemer *et al.*, 2012).

MPC determination. The mutant prevention concentration (MPC) corresponds to the first antibiotic concentration at which no bacterium was recovered when 10^{10} cells were applied to agar plates containing 2-fold increasing antibiotic concentration (Blondeau *et al.*, 2001). Simply, a MPC is an MIC determination with a large inoculum (Mouton *et al.*, 2005). The MPC was determined as described by Blondeau *et al.* (2001) with slight modifications. Briefly, each strain was grown overnight (20 to 24 h) on ten plates of Mueller Hinton (MH) agar supplemented with 5% lysed horse blood and 20 μg/mL β-Nicotinamide adenine dinucleotide at 35 ± 2 °C with 5 ± 2% CO_2. Two mL of MH broth was then added to each plate, spread and pooled to give 20 mL of bacterial suspension. After a centrifugation for 30 min at 5000 ***g***, the supernatant was removed and the remaining pellet was resuspended in 3 mL of MH broth. 0.2 mL of the bacterial suspension (around 10^{10} cells) were spread onto supplemented MH agar plates containing appropriate marbofloxacin concentrations (0.002–8 μg/mL). Plates were incubated at 35 ± 2 °C in air supplemented with 5 ± 2% CO_2 and growth observed at 24 and 48 h. MPC was recorded as the lowest antibiotic concentration that allowed no growth. Due to the complexity of this determination, it was only feasible to carry out this determination in six App and two Hp strains.

PK/PD analysis and Monte Carlo simulation (MCS)

The MCS were performed with Oracle Crystal Ball V.11.1.2.0.00. (Oracle Corporation, Redwood Shores, CA, USA). Two sets of simulations were performed, one for the weaners, using the following formula for the bicompartimental model after intramuscular administration:

$$C(t) = \frac{F \times D \times K01}{V} \times \left(\frac{K21 - \alpha}{(K01 - \alpha) \times (\beta - \alpha)} \times e^{-\alpha t} + \frac{K21 - \beta}{(K01 - \beta) \times (\alpha - \beta)} \times e^{-\beta t} - \frac{K21 - K01}{(\alpha - K01) \times (K01 - \beta)} \times e^{-K01 t} \right)$$

where F is the bioavailability, D is the dose of antibiotic administered, K01 is the absorption rate constant, V is the distribution volume, K21 is an intercompartmental micro-rate constant, α and β are elimination macro-rate constants and t is a given time.

In the case of the fatteners, it was used a monocompartimental model after intramuscular administration:

$$C(t) = \frac{F \times D \times K01}{V \times (K01 - K10)} \times (e^{-K10t} - e^{-K01t})$$

where F is the bioavailability, D is the dose of antibiotic administered, K01 is the absorption rate constant, V is the distribution volume, K10 is the elimination rate constant and t is a given time.

Each simulation set was performed with 10000 simulated PK profiles. The pharmacokinetic values and adjustments used in the models are shown in Table 1 for the bicompartmental model in the case of weaners and in Table 2 for the monocompartmental model in the case of fatteners. The weight was estimated in 25 kg for piglets and 55 kg for fatteners. For the calculations, marbofloxacin concentrations were simulated over 24 h with a step of 0.1 h. $AUC_{0\text{-}24}$ was calculated using the linear trapezoidal mode for each one of the simulated PK profiles.

The following parameters were calculated to foresee the clinical outcome:

a) Probability of target attainment (PTA) (Mouton *et al.*, 2005) in the simulated population taking into account the PK/PD threshold values of $AUC_{0\text{-}24}$/MIC>125 h and C_{max}/MIC>10 for each MIC point calculated ranging in geometric progression from 0.002 to 8 μg/mL.

b) The cumulative fraction of response (CFR) (Mouton *et al.*, 2005). It is the expected population probability to reach the threshold values of $AUC_{0\text{-}24}$/MIC>125 h or C_{max}/MIC>10 taking into account the probability of the MIC strain distribution. A CFR≥90% was considered optimal against a bacterial population, whereas a CFR ≥80% but ≤90% was associated with moderate probabilities of success (Bradley *et al.*, 2003). This is the most practical parameter for practitioners.

Table 1. Pharmacokinetic parameters used in the bicompartmental model for weaners

Parameter	Distribution	Distribution parameters (coefficient of variation%)
Bioavailability (F)	Beta PERT	Min: 0.87; most likely: 0.93; max:0.99
Distribution Volume (Vd)	Log normal	X:1.58; SD: 0.23 (14.6)
K01	Log normal	X:5.85; SD:0.95 (16.23)
K21	Log normal	X:0.18; SD:0.01 (5.55)
α	Fixed Value	X:0.2115
β	Log normal	X:0.05; SD:0.01 (20)

K01, absorption rate constant; K21, intercompartmental micro-rate constant, α, β, elimination rate macro constants, X, average value; SD, standard deviation; coefficient of variation between brackets.

Table 2. Pharmacokinetic parameters used in the monocompartmental model for fatteners

Parameter	Distribution	Distribution parameters (coefficient of variation%)
Bioavailability (F)	Beta PERT	Min: 0.9; Most likely: 0.95; max:1
Distribution volume (Vd)	Log normal	X:1.4; SD: 0.1(7)
K01	Log normal	X:5.06; SD:1.8 (35.57)
K10	Log normal	X:0.05; SD:0.01 (20)

K01, absorption rate constant; K10, elimination rate constant; X, average value; SD, standard deviation; coefficient of variation between brackets.

Furthermore, the following parameters were calculated to predict the likelihood of developing resistances in fluoroquinolones as described in the literature by Cui *et al.* (2006), Drlica and Zhao (2007) and Zhao and Drlica (2008):

c) PTA in the simulated population of the threshold values $AUC_{0\text{-}24}$/MPC>25 h for each MPC point ranging in geometric progression from 0.002 to 8 μg/mL. Other authors pointed slightly lower $AUC_{0\text{-}24}$/MPC as threshold values (Olofsson *et al.*, 2006; Ni *et al.*, 2013) but it was chosen a value of $AUC_{0\text{-}24}$/MPC>25 h as a worst case scenario.

RESULTS

MPC results

MPC were determined against six strains of App with a MIC of 0.03 μg/mL (n = 3) and 0.06 μg/mL (n = 3). Two strains of Hpp with a MIC of 0.015 and 0.03 μg/mL were also tested. MPC results are shown in Table 3.

For all App strains (MIC of 0.03 to 0.06 μg/mL), the MPC were comprised between 0.12 to 0.5 μg/mL, which corresponded to 2- to 8-fold MIC. No mutants were able to grow at a concentration above 0.5 μg/mL even in strains with reduced susceptibility. In the case of Hp, MPC were equal to 0.015–0.06 μg/mL (1- to 2-fold MIC).

PK simulation

The simulated PK profiles, using the mean, maximum and minimum values obtained from simulations, are presented in Fig. 1. The mean clearance values that were calculated from simulations (Tables 1 and 2) were 0.12±0.11 L/kg/h (coefficient of variation: 91.6%) for fatteners and 0.09±0.02 L/kg/h (coefficient of variation: 22%) for weaners, respectively. These results are in agreement with a clearance value of 0.12±0.02 L/kg/h described by Ding *et al.* (2010). Furthermore, Schneider *et al.* (2014) described clearance values of 0.092 and 0.079 L/kg/h in piglets and fatteners, respectively.

Table 3. Mutation prevention concentration (MPC) and minimum inhibitory concentration (MIC) of six *App* and two *Hp* strains

Isolate details		MIC (μg/mL)	MPC (μg/mL)	
Isolate number	Species	Agar dilution	Duplicate 1	Duplicate 2
1	*A. pleuropneumoniae*	0.03	0.25	0.12
2	*A. pleuropneumoniae*	0.03	0.25	0.12
3	*A. pleuropneumoniae*	0.03	0.12	0.12
4	*A. pleuropneumoniae*	0.06	0.25	0.12
5	*A. pleuropneumoniae*	0.06	0.5	0.12
6	*A. pleuropneumoniae*	0.06	0.25	0.25
7	*H. parasuis*	0.015	0.015	0.03
8	*H. parasuis*	0.03	0.06	0.06

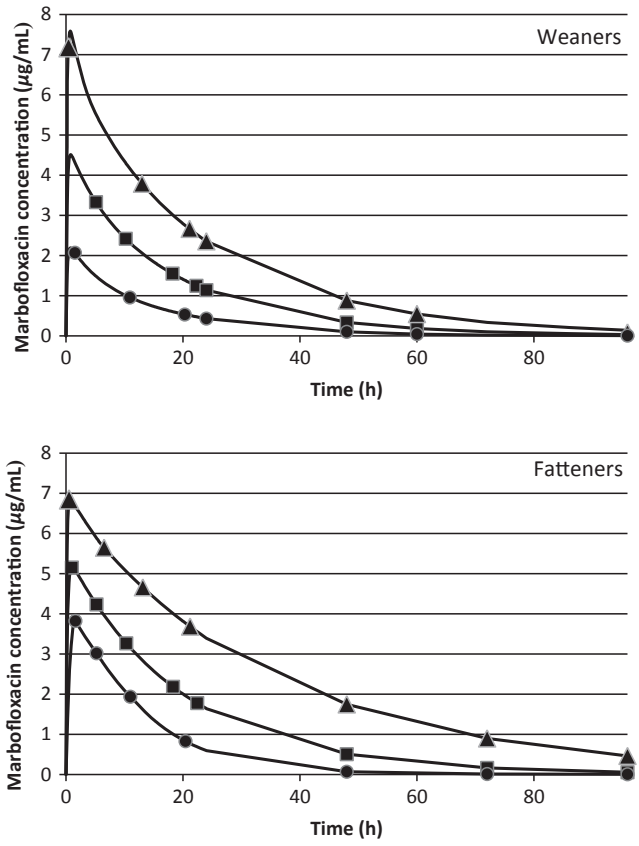


Fig. 1. Pharmacokinetic profiles depicted using the values of the mean (square), maximum (triangle) and minimum (circle) values obtained from the simulations.

Clinical outcome

The probability of target attainment (PTA) of the simulated pig population taking into account a threshold value of $AUC_{0\text{-}24}$/MIC>125 h for a MIC range from 0.002 to 8 μg/mL of App and Hp strains to marbofloxacin is shown in Fig. 2. In this case, the PTA is 100% at the three posology regimen studied for a MIC lower than 0.06 μg/mL for both bacteria (App and Hp) and this value is 0% for MIC values higher than 1 μg/mL in both cases. This PTA value would decrease from 100% to a value lower than 40% in weaner pigs at MIC values of 0.12, 0.25 and 0.5 μg/mL for both bacteria at a dose of 2, 4 and 8 mg/kg bw of marbofloxacin, respectively. In the case of fatteners, the PTA is 100% at the three posology regimen studied for a MIC value lower than 0.12 μg/mL for both bacteria (App and Hp) and 0% for MIC values higher than 1 μg/mL for both pathogens. Finally, the PTA value would decrease from 100% to a value of 0% in fattener pigs at MIC values of 0.25, 0. 5 and 1 μg/mL for both bacteria at a dose of 2, 4 and 8 mg/kg bw of marbofloxacin, respectively (Fig. 2).

The probability of target attainment (PTA) of the simulated pig population taking into account a threshold value of C_{max}/MIC>10 for App and Hp strains to marbofloxacin is shown in Fig. 3. The results obtained are almost the same as previously described (Fig. 2) for the other surrogate marker ($AUC_{0\text{-}24}$/MIC>125 h) with the particularity that the PTA value would decrease from 100% at the same MIC points in fattener (60–80%) than in weaner pigs (20–30%) at 0.12, 0.25 and 0.5 μg/mL for both bacteria at a dose of 2, 4 and 8 mg/kg bw of marbofloxacin, respectively.

The cumulative fraction of responses (CFRs) of the simulated pig population for the three posology regimens according to their probability of MIC strain distribution are shown in Table 4. The same CFRs values were obtained using $AUC_{0\text{-}24}$/MIC or C_{max}/MIC as surrogate markers. The CFR value was higher than 91% and 80.5% for App and Hp for all the studied posology regimens, respectively. Overall, fatteners showed a slightly better theoretical clinical outcome (CFR value) than weaners for both bacteria in all the studied posology regimens reaching the best result at the dose of 8 mg/kg of marbofloxacin in weaners (above 96% for App and 85% for Hp) and fatteners (97% for App and 88% for Hp).

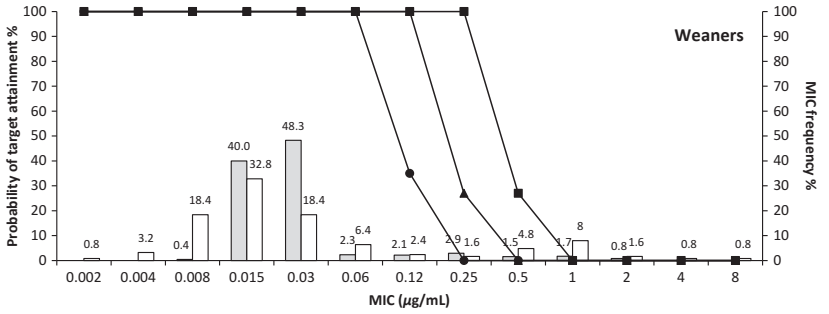
Appearance of resistances

The probability of target attainment (PTA) of the simulated pig population taking into account a threshold value of $AUC_{0\text{-}24}$/MPC>25 h to avoid the appearance of antimicrobial resistance is shown in Fig. 4.

The PTA of the threshold values for preventing antimicrobial resistance of $AUC_{0\text{-}24}$/MPC>25 h across different MPC points (Fig. 4) clearly show that the generation of antimicrobial resistance up to a MPC value of 0.25, 0.5 will be avoided, and 1 μg/mL for both bacteria at a dose of 2, 4 and 8 mg/kg bw of marbofloxacin in weaners, respectively. However, this generation will be probably avoided up to a MPC value of 0.5, 1 and 2 μg/mL for both bacteria at a dose of 2, 4 and and 8 mg/kg bw of marbofloxacin in fatteners, respectively. The same analysis was carried out taking into account the effect of the first dose (data not shown) and it was not observed any difference in comparison with the results obtained for the whole posology regimen.

DISCUSSION

It is widely accepted that drugs are well-designed to cover most of the bug strain population according to PK parameters determined in preclinical studies in the target species. However, it will be very interesting to analyse the probability of success in any antibiotic treatment and in the generation of antimicrobial resistance taking into account the pharmacokinetic and pharmacodynamic variability observed for the pig and micro-organisms, respectively. This type of analysis could be even more relevant if it is taking on board the presence of a population of different strains of the same micro-organism in one animal (Lowe *et al.*, 2012; Vilalta *et al.*, 2012). Finally, this type of analysis could be relevant to foresee the clinical efficacy and the generation of antimicrobial resistance in a dynamic



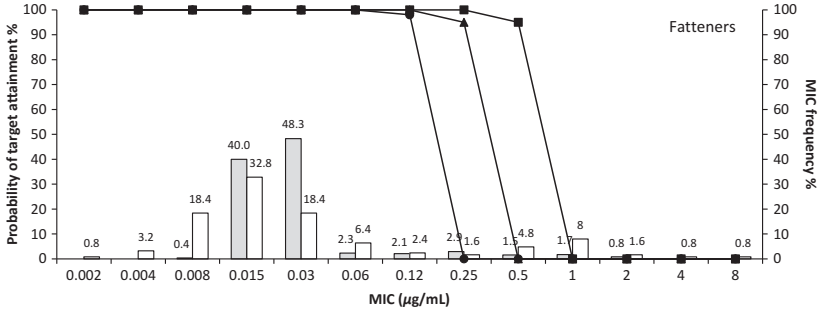
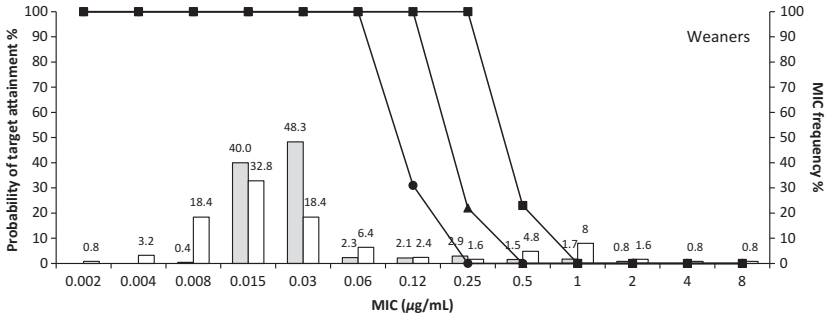


Fig. 2. Graphical representation of the probability of target attainment of the threshold value of $AUC_{0\text{-}24}$/MIC>125 of the simulated values according to each MIC point for weaners and fatteners in the three posology regimens: 2 mg/kg bw (circles), 4 mg/kg bw (triangles) and 8 mg/kg bw (squares) and MIC distribution of marbofloxacin, expressed as strain percentage, against *A. pleuropneumoniae* (grey bars) and *H. parasuis* (white bars).



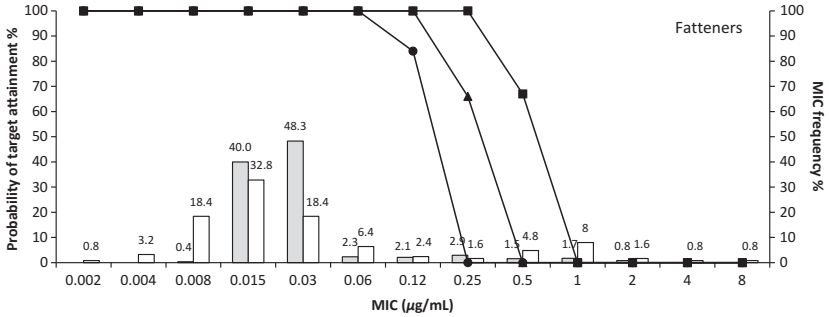


Fig. 3. Graphical representation of the probability of target attainment of the threshold value of C_{max}/MIC>10 of the simulated values according to each MIC point for weaners and fatteners in the three posology regimens: 2 mg/kg bw (circles), 4 mg/kg bw (triangles) and 8 mg/kg bw (squares) and MIC distribution of marbofloxacin, expressed as strain percentage, against *A. pleuropneumoniae* (grey bars) and *H. parasuis* (white bars).

population of micro-organism whose pharmacodynamic properties are continuously evolving.

In this study, PK/PD simulations were performed to evaluate three different posology regimens of marbofloxacin, taking into account the antimicrobial susceptibility of App and Hp and the pharmacokinetic variability of two different groups of pigs, weaners and fatteners. The probability of clinical success was evaluated through the use of the $AUC_{0\text{-}24}$/MIC and C_{max}/MIC index, while the risk of emergence of mutants was evaluated through the use of $AUC_{0\text{-}24}$/MPC index as surrogate markers. Although the same procedures used here have been applied on several occasions to calculate the usefulness of therapeutic strategies in human medicine (Isla *et al.*, 2011; Cao *et al.*, 2013; Goff & Nicolau, 2013), there are some limitations that should be discussed. PK/PD calculations were based on the total drug concentration on serum. It could be assumed that marbofloxacin concentration in the site of action, lung and bronchial secretions, was at least very similar to that observed in serum due to the high bioavailability, low protein binding and tissue distribution reported for fluoroquinolones (Martinez

Table 4. Cumulative fractions of response (CFR) (%) of the threshold values of $AUC_{0\text{-}24}$/MIC>125 h and C_{max}/MIC>10 (between brackets) for the simulated populations of weaners and fatteners when crossed with the MIC distribution probability of the different posology regimens: 2 mg/kg bw, 4 mg/kg bw and 8 mg/kg bw of marbofloxacin

	A. pleuropneumoniae	*H. parasuis*
Weaners		
2 mg/kg bw	91.12 (91.63)	80.52 (80.78)
4 mg/kg bw	93.99 (93.86)	83.19 (82.65)
8 mg/kg bw	96.37 (96.33)	85.1 (85.14)
Fatteners		
2 mg/kg bw	93 (92.92)	82.01 (82.27)
4 mg/kg bw	95.72 (95.08)	83.96 (83.32)
8 mg/kg bw	97.43 (97)	88.49 (87.42)

et al., 2006). Hence, for example, Messenger *et al.* (2012) found that the tissue penetration ratio ($AUC_{tissue}/AUC_{plasma}$) of enrofloxacin in the pleural cavity in pigs was 1.40 ± 0.35 and Bimazubute *et al.* (2009) described a value of 1.26 for the same ratio in the nasal secretions. In conclusion, it seems very reasonable to use the available concentration observed in plasma to foresee the clinical efficacy of this antibiotic. Other clear limitation of this study is that PK parameters used in this research work (from a limited number of animals) could not represent the real interindividual variability of the PK parameters in the targeted animal population not only in healthy animals but also in sick ones. Thus, as an alternative to the previous point, when experimental population data are lacking, the simulations can be performed using '*a priori*' values of the interindividual variability that are 'reasonably' high enough to fit with the real situation. This point has been accomplished in this research work. Thus, the coefficients of variation for marbofloxacin clearance, used during the simulation for fatteners and weaners, were 90 and 20%, respectively. The variability used for this parameter is higher than the previously published by other authors for this molecule (Ding *et al.*, 2010; Schneider *et al.*, 2014). In conclusion, authors believe that the variability used is reasonable enough and it should not be a limitation for the extrapolation of the results obtained to the whole pig population.

This study shows slight differences between the foreseen clinical outcome in late weaners and early fatteners in spite of the differences observed in the pharmacokinetic in both age groups (Schneider *et al.*, 2014) when the effect of the usual posology regimens in use under field conditions (2 mg/kg bw three times each 24 h, 4 mg/kg bw twice each 48 h and 8 mg/kg bw in one single shot) was compared. In this sense, it has to be taken into account that some of the veterinary drugs are not designed to reach a steady-state. Thus, many drugs are designed to get their clinical outcome in one, two or three doses at most with the exemption of the administered orally through water or food. Therefore, it seems that a 'classical' steady-state for many drugs is not reached. An equivalent parameter of the classical AUC/MIC or AUC_{ss}/MIC for those drugs which do not reach the steady-state would be the $AUC_{0\text{-}\infty}$/MIC as pointed out by some authors (Toutain *et al.*, 2007). In this research, the period between 0 and 24 h for the three posology regimes (2, 4 and 8 mg/kg bw) was studied because it seems that marbofloxacin exerts its higher effect on bacteria during this period of time according to an *in vitro* dynamic test carried with strains of *Mannheimia haemolytica* and *Pasteurella multocida* (Vallé *et al.*, 2012). The authors have not found any other information in veterinary medicine comparing different posology regimes using the foreseen effect during the first 24 h. It is clear that further research should be carried out in this matter. On the other hand, other factors besides single or

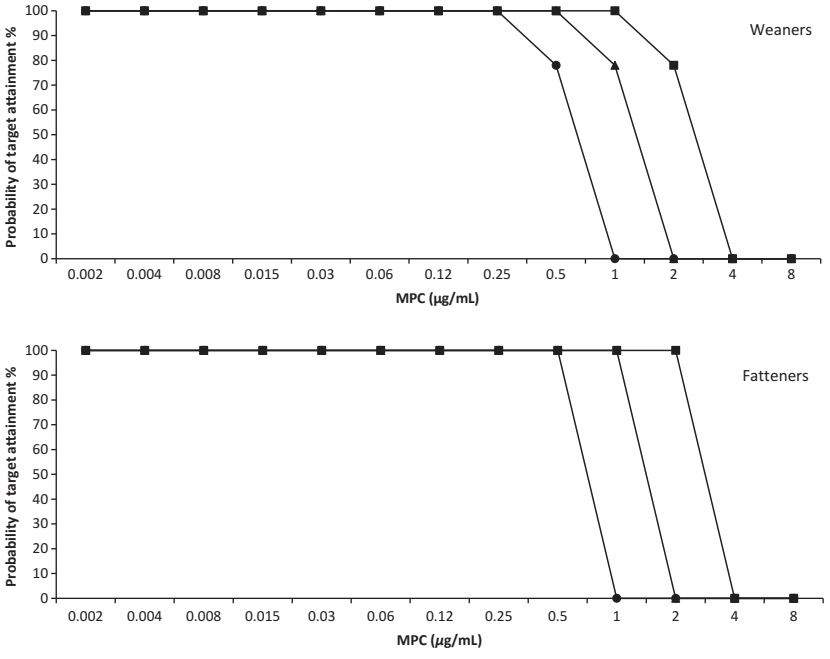


Fig. 4. Probability of target attainment of the threshold value of $AUC_{0\text{-}24}$/MPC>25 for weaners and fatteners in the three posology regimens: 2 mg/kg bw (circles), 4 mg/kg bw (triangles) and 8 mg/kg bw (squares).

multiple doses should be considered when setting the treatment such as the early or late treatment and consequently the size of the bioburden at the site of infection (Ferran *et al.*, 2011) and the MIC or MPC of the offending pathogen. Although, some studies corroborate the efficacy of the multiple dose regimens to eradicate or control a pathogen (Aliabadi & Lees, 2002; Sidhu *et al.*, 2011; Vilalta *et al.*, 2011), the concept of an aggressive early treatment seems to be more suitable to treat infections, reach the PK/PD targets for fluoroquinolones and prevent mutations (Martinez *et al.*, 2012). Our results agree with the literature because the best clinical outcome was foreseen for the 8 mg/kg bw in one single shot. Moreover, similar results were obtained using $AUC_{0\text{-}24}$ or C_{max} as surrogate markers of clinical outcome reinforcing that both parameters could be used for this purpose as it has been published previously in the literature (Drusano *et al.*, 1993; Mckellar *et al.*, 2004; Lees, 2013; Papich, 2014).

Similar values were obtained in the three posology regimens for App and Hp when the effect of preventing resistances was simulated using the marker $AUC_{0\text{-}24}$/MPC. Thus, it would seem quite reasonable to use equally any of the three treatments previously commented. Different opinions can be found in the literature about this topic, while some authors pointed that the single high-dose shot of marbofloxacin would reduce the amplification of resistances (Vallé *et al.*, 2012), other authors concluded that the fractionated dose of the same antimicrobial would be more beneficial to prevent those resistances (Kesteman *et al.*, 2009). Monte Carlo simulations had not taken into account other factors that could lead to the amplification of resistant subpopulations such as the size of the bioburden at the infection site (Ferran *et al.*, 2011), as commented previously, biofilm formation or the effect on other bacterial populations, as the gut flora (Kesteman *et al.*, 2010). Despite these limitations, it is assumed that an early treatment of a highly concentrated drug is more likely to minimize and prevent the amplification of resistances avoiding the growth of the bioburden that could lead to high bacterial density scenario where mutations are more likely to occur. In our case, the 8 mg/kg bw of marbofloxacin in one shot would reach concentrations with a high probability of being above the MPC and would be a reliable option when it comes to prevent the amplification of resistances, at least in the target site. Finally, the results obtained in connection with the generation of antimicrobial resistance should be considered preliminary due to the low number of strains included in the simulation process. This observation is even more relevant in the case of Hp strains where the MPC value is equal or slightly higher than the MIC value. For this reason, additional studies with a higher number of strains are compulsory in order to confirm the obtained results.

Practitioners usually have to start a treatment against App and Hp without knowing the MIC of the causative pathogen. Thus, the CFR calculated in this study could be a good way to estimate the potential for a positive clinical outcome in any herd. To our knowledge, this is one of the first scientific publications in the veterinary field where this approach is carried out. In the future, it could be a good way to select the best antimicrobial to treat an infection following a prudent use of these drugs. It is important to keep in mind that MIC probability distribution of a determined pathogen may vary between countries and regions and even time. Taking into account the MIC distribution provided by Vetoquinol marbofloxacin MIC surveillance program (Giboin *et al.*, 2012), it could be assumed that a marbofloxacin treatment would achieve a CFR of more than 90% (ranging from 91 to 97 depending on the dose) against App and between the range 80–90% against Hp. Although, the CFR for Hp is lower than the App CFR, marbofloxacin would be a reliable option when it comes to treat infections caused by these pathogens. It would have been very interesting to assess CFR for the prevention of resistances, but there is not enough information about MPC and its probability frequencies distribution.

Veterinarians usually treat large populations of animals without knowing the MIC of offending bacteria. Although, the use of CFRs is new in veterinary medicine, it is a used tool in human medicine to compare treatments and foresee clinical failure. Knowing the CFRs of the antimicrobials and bugs should be a good tool to select a treatment and to predict possible outcomes. Thus, PK-PD analysis and Monte Carlo simulations are highly valuable techniques to maximize the favourable result of a therapy but further studies are needed to address this matter.

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